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EFFECT OF THE POSTERIOR PITUITARY ANTIDIURETIC HORMONE ON THE MORPHOLOGY OF THE ADRENAL CORTEX IN WATER INTOXICATION

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EFFECT OF THE POSTERIOR PITUITARY ANTIDIURETIC HORMONE
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ABSTRACT

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The effect of water intoxication on the adrenal cortex is profoundly altered by simultaneous administration of posterior pituitary antidiuretic hormone, which produces more marked cortical hyperplasia, principally in the glomerular zone together with heavy depletion of lipids. The results confirm that a functional correlation exists between the posterior pituitary and the adrenal cortex in terms of water metabolism.

AUTHOR

It has been known for some time that chronic adrenocortical insufficiency is accompanied by serious disturbances in water and electrolyte metabolism, with dehydration or exsiccosis, hypodipsia, oliguria, increased urinary sodium elimination and slight diuretic response to an acute water load. Experimentally, it has been found that in the adrenalectomized animal in the same way as can be observed in human pathology in Addison's disease, the water introduced is slowly eliminated (Silvette and Britton) and diuresis is not stimulated by administration of water, glucose or hypotonic solutions (Ringler, Swingle et al; Gaunt et al.). In contrast, diuresis with a water load is increased by adrenocortical extract (Osborn and Eversole) and by desoxycorticosterone (Seyle and Basset; Osborn and Eversole) which when administered for long periods of time may produce a polyuric syndrome similar to diabetes insipidus (Kuhlmann et al, Mulinos et al.). /275

The antidiuretic hormone of the posterior pituitary is the second important factor regulating water and salt metabolism. Its action, in some respects antagonistic to the adrenal cortex and desoxycorticosterone, has led some to claim that normal water and electrolyte balance is maintained by the opposed effect of the adrenal cortex and the posterior pituitary (Britton and Kline). Desoxycorticosterone as well as inhibiting tubular reabsorption of water (Gaunt et al.) promotes intense glomerular filtration and retention of chloride and sodium (Harrop, Swingle et al.), while pituitary extracts tend to increase water reabsorption, reduce water filtration (Villa and Gavazzeni)and raise the level of salt excretion (Little, Wallace et al.). The result is that all the changes induced in the adrenal cortex-posterior pituitary system are manifest in a disturbed water and electrolyte balance, as a whole, which clinically may culminate either in a polyuric picture in diabetes insipidus or in a syndrome of Addison's oliguria. Close functional interdependence of the posterior pituitary and the adrenal cortex is also indicated by the investigations of Birnie et al. and Jenkins and Birnie, on the presence of posterior pituitary antidiuretic hormone in the blood of animals and humans and its increase in the blood and urine of animals after adrenalectomy and the restoration of normal values and regulation of diuresis with administration of desoxycorticosterone. /276

The effect of the adrenal cortex on water-salt metabolism clearly demonstrated by many clinical and experimental investigations is brought out even more clearly with the forced water load test. In the normal animal administration of considerable amounts of water elicits a marked diuretic response and the rapid elimination of the excess liquid makes it more difficult for manifestations of water intoxication to appear (Swingle, Hays et al). In contrast, in the adrenalectomized animal which, together with deficient diuresis, displays enhanced "sensitivity to hydration" (Swingle, Remington et al.), the acute water load soon results in a toxic picture with excitability, increased salivation, convulsions and opisthotonus (Swingle, Parkinson et al.). Cortical extracts and desoxycorticosterone in such cases as well as rendering normal the diuretic capacity of the adrenalectomized animal, impede the appearance of signs of intoxication due to excess water.

Further, in the normal animal put on a forced water load and simultaneously treated with antidiuretic hormone, diuresis is seriously obstructed and elimination of water via the kidneys instead of rapidly reaching maximum values as it normally does, does not differ, for a certain time, from the mean values recorded in the adrenalectomized animal. Only on cessation of the antiduiretic effect is there critical urinary excretion capable of eliminating the excess water.

Therefore, on the basis of these premises and, in particular, bearing in mind the functional antagonism between the adrenal cortex and posterior pituitary in terms of water-salt metabolism, we felt it to be of some interest to see whether in the animal on a forced water load and treated with the posterior-pituitary antidiuretic hormone it is possible to induce in the suprarenals definite changes in cortical morphology and histochemistry.

Material and Methods

For the experiment we used white adult female rats weighing 200 - 280 g. The animals, well hydrated and kept on a mixed diet were divided into two groups. The first group was given a forced water load. In the second, the water load was combined with treatment with posterior pituitary hormone. The water load with 0.9% saline was administered twice a day at an interval of 8 hr and during the test the animals were not fed. Food was given in the evening after the second load. The amount of liquid which on the first administration with stomach tube was 5 % of body weight was progressively and rapidly increased within two days to 10 % body weight and this amount maintained throughout the experiment. In the group which received posterior pituitary hormone, the daily dose of pituitary extract was 5 IU per animal in two equal doses, injected immediately after the water load. In preliminary checks 2.5 IU pituitary extract injected subcutaneously in normal animals well hydrated and on an acute water load via the gastric route produced marked water retention lasting 5 hr after administration. /277
The animals of both groups sacrificed after a time interval of 15 - 20 days from the start of the experiment were weighed on an empty stomach and killed by decapitation some 8 hr after the last water load. The two adrenal glands, the pituitary and the heart were removed and weighed. One suprarenal per rat was fixed in 10 per cent neutral formalin for at least 24 hr. After fixing and washing in running water it was sectioned with a freezer-microtome and sections 10 μ thick were used to study the histochemical reactions by methods proposed for study of cortical lipids (Staining with Sudan III, Sudan black and Schiff's reagent, and by the method of Liebermann-Schultze). In the other unstained sections some of which were treated with cold acetone for half an hour, we estimated birefringence and the presence of birefringent acetone-soluble substances. Some of these tests show general lipid accumulation in the adrenal cortex, others are closely related to the content of sterols (cholesterol and cholesterol esters) which are thought to represent the true functional lipids of the gland or precursors of specific cortical hormones. The other suprarenal and pituitary, after being fixed in 10 % formalin, washed and embedded in paraffin, were sectioned and stained with hematoxylin-eosin after recording the variations in width in the area of the suprarenal glomerular zone of the two groups of animals, the changes

in this zone were measured in the hematoxylin-eosin preparations and expressed in microns.

For statistical evaluation of the results the table gives for some media, the value of the standard error (ϵ) calculated from the formula $\sqrt{\frac{S\,d^2}{n(n-1)}}$ and Student's "t" calculated from the formula

$$\frac{\bar{x} - \bar{y}}{\sqrt{\frac{S\,x^2 + S\,y^2}{n_1 + n_2 \cdot 2} - (\frac{1}{n_1} + \frac{1}{n_2}}}$$

used for establishing the significance of the differences in small experimental groups. Differences for which "t" exceeded 2.2 were considered statistically significant.

Experimental Results

The animals treated only with a forced water load 15 - 21 days from the start of the experiment showed little loss of body weight, while rats with a forced water load and pituitary extract showed a more marked fall. Table 1 gives the variations in the average body weight for both groups at the end of the experiment.

The mean relative weights of the suprarenal glands, the pituitary and the heart of the animals of both groups are also indicated in Table 1. The weight of the pituitary and the heart of the animals on a water load was normal for rats of this age and body weight; that of the suprarenals was slightly above normal. The weight of the suprarenals of the animals on a water load and pituitary extract was, in contrast, much higher, while that of the /278

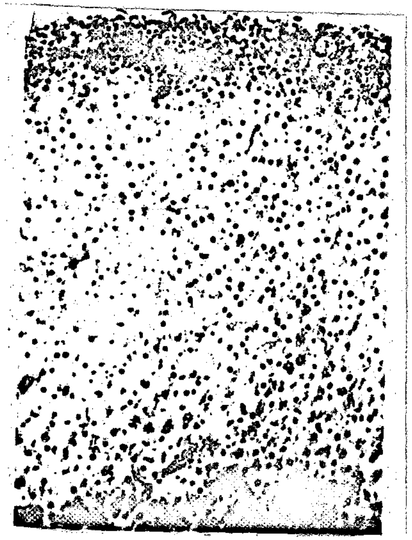

Fig. A.

Adrenal cortex of the rat subjected to water intoxication and treatment with posterior pituitary hormone. Marked widening of the glomerular zone. Fascicular zone with dark cells without vacuoles. Staining with hematoxylin-eosin. Magnification X 135.

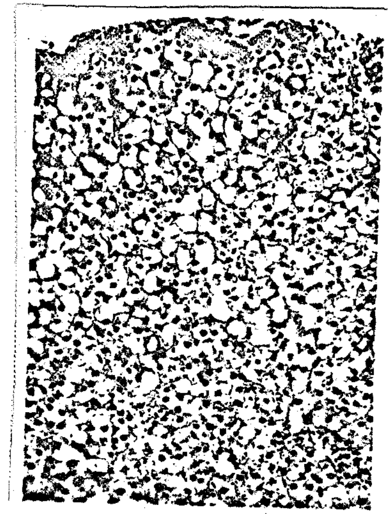

Fig. B.

Adrenal cortex of hyperhydrated rat. Glomerular zone of normal width. The cell elements of the fascicular zones show large vacuoles. Staining with hematoxylin-eosin. Magnification X 135.

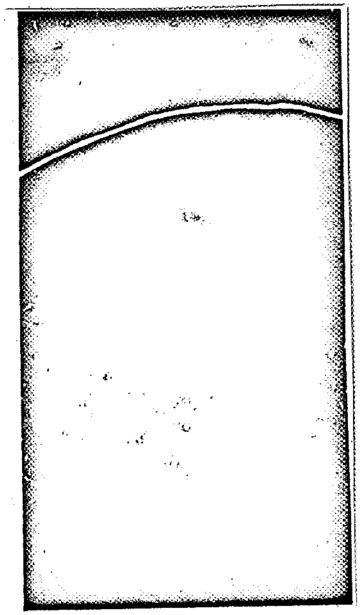

Fig. C.

Examination of birefringence in polarized light in unstained sections of the suprarenals of the rat put on a forced water load and treated with post pituitary hormone. Almost complete absence of birefringent substances in the glomerular and external fascicular zones, fine granules in the internal fascicular. Magnification X 65.

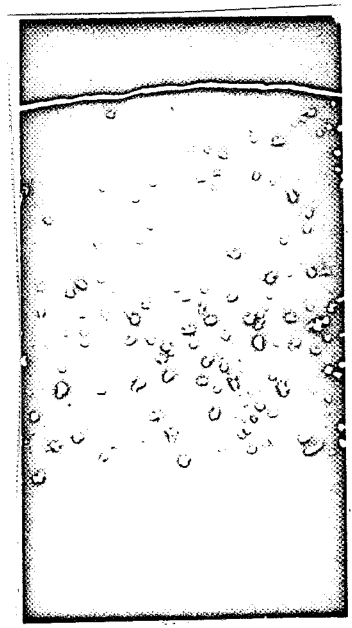

Fig. D.

Examination of birefringence in polarized light in unstained sections of the suprarenals of the hyperhydrated rat. Reduced birefringent substance in the glomerular and external fascicular zones; coarse granules in the internal fascicular zone. Magnification X 65.

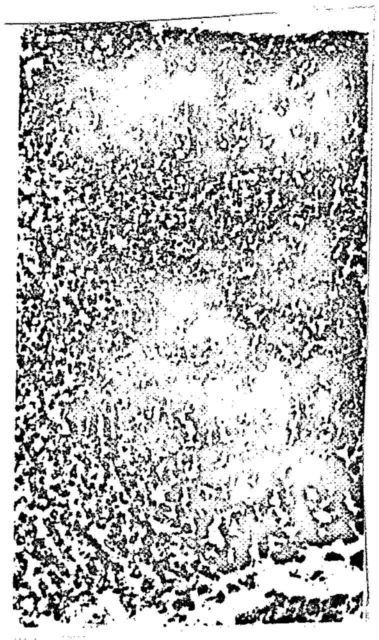

Fig. E.

Sections of suprarenals of hyperhydrated rat treated with posterior pituitary hormone. Staining with Sudan black. Marked fall in lipid level in cortex. Magnification X 65.

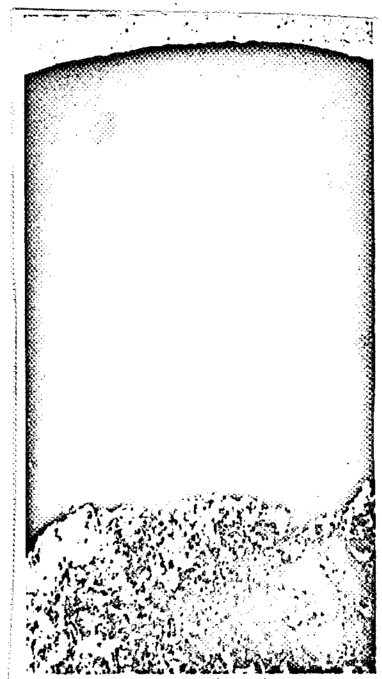

Fig. F.

Suprarenals of water intoxicated rat. Staining with Sudan black. Marked increase in the lipid content in all cortical zones. Magnification X 65.

TABLE 1

No. of rats	Treatment	Initial weight g	Final weight g	Weight difference (+,-)	Suprarenals mg/100 g $\pm\epsilon$	"t"	Pituitary mg/100 g $\pm\epsilon$	Heart mg. % $\pm\epsilon$
15	Water load	237	218	-19	3.38 $\pm$ $\pm$ 2.9		6.0 $\pm$ $\pm$ 0.4	452 $\pm$ $\pm$ 31
15	Water load + pituitary extract	251	214	-37	47.2 $\pm$ $\pm$ 3.4	3.6	6.2 $\pm$ $\pm$ 0.6	455 $\pm$ $\pm$ 29

pituitary and heart showed insignificant differences compared with those of animals only on a water load.

The adrenal cortex of the animals on a forced water load showed the following changes in the hematoxylin-eosin preparations: the glomerular zone was a little irregular and of normal width, being 28 - 35 μ. It was formed of rather small closely packed cells often containing vacuolar formations. The fascicular zone was slightly wider than normal, made up of cells of medium size clearly shown up by the presence of large intracellular vacuoles, while the reticular zone was represented by small juxtamedullary cell elements of medium size and without vacuoles. Histochemical examination showed diffuse large-drop sudanophilia in all cortical layers, especially in the external fascicular zone. Birefringence was weak in the glomerular and external fascicular zones and took the form of spherocrystals and rather coarse acetone-soluble crystals in the internal fascicular zone. The Schiff test was positive and of almost uniform intensity throughout the cortical zones and the Schultze test was positive in the glomerular and fascicular zones.

In the animals of the second experimental series put on a forced water load, together with posterior pituitary hormone, the glomerular zone greatly increased in width and on the 15 to 20th day of the experiment measured 60 - 90 μ. Such an increase was essentially due to hyperplasia and hypertrophy of the cell elements forming the glomerular zone. The fascicular zone also widened. The cells of the glomerular and fascicular zones were of a dark appearance without any vacuolar formations. The cortical sinusoids were wide, dilatated and filled with blood, and often we observed zones of subcapsular nodular hyperplasia. Histochemically, the lipid content was greatly reduced and the sudanophilic drops sparse and irregularly distributed. The reduction in the sudanophilic material was greater in the glomerular and external fascicular zones. The birefringent particles were very fine or absent in the glomerular and external fascicular zones with very fine crystals in the internal fascicular zone. The Schiff reaction was also much weaker in all cortical zones and especially in the glomerular and external fascicular and in the Schultze test the cholesterol level was very moderate and limited to the more external portion of the glomerular zone while the fascicular zone showed slight irregularly distributed accumulations.

Remarks and Conclusions

In the present investigation aimed at establishing whether direct relations exist between the morphology of the adrenal cortex and water and salt metabolism, groups of intact rats were put on a forced water load (water intoxication). In order to induce profound changes in water and salt metabolism in a group of animals hyper-hydration was combined with posterior pituitary antidiuretic and chloruretic treatment. The purpose of the present study was to com- /279
pare changes if any in the adrenal cortex of the two experimental groups of animals and to see whether these are well differentiable and note their relation to stimuli of metabolism. Our investigation was also concerned with the stress effect of water intoxication and the changes in the suprarenals were also interpreted in relation to this unspecific stimulus.

Taking the results as a whole it follows that prolonged hyper-hydration does not produce marked changes in the corticosterol level nor obvious changes in the cytology of the various zones. Thus, the anatomic picture even allowing for the increase in the weight of the suprarenals occurring in these conditions is essentially the same as that observed in the adaptive phase following heavy repeated "stress" (stage or type 4 of Sayers and Sayers). In contrast, in the animal exposed to hyperhydration and treated with pituitary extract, together with a marked lipid loss and a heavy increase in the total weight of the gland we observed pronounced widening of the glomerular zones with sites of subcapsular nodular hyperplasia. In this case then as well as the changes usually observed during unspecific continuous and intense stress with absence of the adaptive phase (stage or type 3 of Sayers and Sayers) it is also possible to notice in the glomerular zone special anatomic changes characterized notably by increase in the thickness of the zone through hyperplasia and hypertrophy of the cell elements forming it.

Two hypotheses may be suggested to explain this morphologic finding: namely, that such changes are linked with intense and prolonged unspecific cortical stimulation or we may accept the idea that the glomerular zone is the source of production of mineralocorticoid hormones strictly related to the profound metabolic water and salt disorder produced by water intoxication and posterior pituitary extract.

The latter hypothesis would appear to be supported both by our present knowledge of the antagonism between adrenal desoxycorticoids and posterior pituitary hormone (Silvette and Britton, Rommelt, Sartorius and Roberts) and by our knowledge of the action of desoxycorticosterone, and prototype of mineralocorticoid hormones and the considerable effects which may be obtained with such substances in the disturbed water and electrolyte balance in an Addison's patient.

Bibliography

1. Bergner, G. E. and Dean, H. W. Endocrinol., 43, 240, 1948.
2. Birnie, J. H., Jenkins, R., Eversole, W. J. and Gaunt, R. Proc. Soc. Biol. Med., 70, 83, 1949.
3. Boss, W. R., Birnie, J. H. and Gaunt, R. J. Clin. Endocrin. I, 658, 1948. id., Endocrin., 46, 307, 1950.
4. Britton, S. W. and Kline, R. F. Am. J. Phys., 133, 503, 1941.
5. Carnes, W. H., Ragan, G., Ferreira, J. W. and O'Neill, J. Endocrinol. 29, 144, 1941.
6. Castello, L. Ormonologia, 9, 1, 1950.
7. Castillo Del, E. B. and Rapela, C. E. Rev. Soc. Arg. Biol. 21, 339, 1945.
8. Cavallero, C. Rass. Clin. Scient., 7, 204, 1950.
9. Deane, H. W., Shaw, J. H. and Greep, R. O. Endocrinol., 43, 133, 1948.
10. Dempsey, E. W. and Wislocki, G. B. Physiol. Rev., 26, 1, 1946.

11. Gaunt, R., Liling, M. and Cordsen, M. Endocrinol., 37, 136, 1945.
12. Harrop, G. A. and Thorn, G. W. J. Exp. Med., 65, 757, 1937.
13. Kuhlman, D., Ragan, C., Ferrebee, J. W., Atchley, D. W. and Loeb, R. F. Science, 90, 496, 1939.
14. Jenkins, R. and Birnie, J. R. Anat. Rec.: 103, 127, 1949.
15. Little, J. M., Wallace, S. L., Wanthey, E. C. and Anderson, G. A. Am. J. Phys., 151, 174, 1947.
16. Mulinos, M. G., Springarn, C. L. and Lojkin, M. E. Am. Phys., 135, 102, 1941.
17. Osborn, C. M. and Eversole, W. J. Fed. Proc., 8, 122, 1949.
18. Ringler, R. Klin. Wschr., 14, 1, 1935.
19. Sayers, G. and Sayers, M. A. Rec. Progr. Horm. Research., 2, 81, 1948.
20. Sartorius, O. W. and Roberts, K. Endocrinol., 45, 273, 1949.
21. Sartorius, O. W., Rommelt, J. C. and Pitts, R. F. J. Clin. Investig., 28, 423, 1949.
22. Selye, H. and Basset, L. Proc. Soc. Exp. Biol. Med., 45, 272, 1940.
23. Silvette, H. and Britton, S. W. Am. J. Physiol., 104, 399, 1933, id., Am. J. Physiol., 123, 630, 1938.
24. Swingle, W., Parkinson, W. M., Taylor, A. R. and Hays, H. W. Am. J. Physiol., 119, 557, 1937.
25. Swingle, W. and Remington, J. W. Physiol. Rev., 24, 89, 1944.
26. Swingle, W., Remington, J. W., Hays, H. M. and Collings, M. D. Endocrinol., 28, 531, 1941.
27. Tronchetti, F. Folia Endocrin., 3, 93, 1950.
28. Villa, L. and Gavazzeni. Policlinico (Sez. med)., 39, 1932.

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